

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: Gregory T. Bleck, et al.

Serial No.: 10/759,315

Group No.: 1633

Filed: 1/16/04

Examiner: Popa

Entitled: **PRODUCTION OF HOST CELLS CONTAINING MULTIPLE
INTEGRATING VECTORS BY SERIAL TRANSDUCTION**

FOURTH DECLARATION OF DR. GREGORY BLECK

I, Dr. Gregory Bleck, state as follows:

1. My present position is Senior Director, Cell Line Engineering, Catalent Pharma Solutions-Middleton, WI.
2. I am an inventor of the above referenced patent application.
3. In the Office Action dated July 17, 2009, the Examiner addressed my previous Declarations by stating:

Applicant argues that the Second Bleck Declaration establishes that one of skill in the art would not extrapolate the data of Mathor et al. to conclude that making cell lines with genomes comprising greater than 20 integration events was desired or feasible. The Second Bleck Declaration was previously presented and addressed. Furthermore, apart from an argument, Applicant and his Declaration did not provide any evidence indicating that obtaining host cells with genomes comprising greater than 20 integrated viral vectors was not feasible before the instant invention was made. It is noted that Table 1 (to which Applicant refers to) shows the result obtained with only one keratinocyte clone for each 8 and 15 integration events. Such results cannot be extrapolated to all clones. The Examiner provided evidence to support this statement and it is not clear why Applicant argues that such a statement is sheer speculation and an unsupported extrapolation of the data. It is well established in the art that retroviral insertion is random and that expression level is dependent on the insertion sites (see Mathor et al., of record, p. 10376, column 1; Liu et al., of record, Anal Biochem, 2000, 280: 20-28, Abstract, p. 21, column 1; Stamps et al., of record, Int J Cancer, 1994, 57: 865-874, Abstract, p. 868, column 1, p. 869, Fig. 2). It is noted that Applicant did not indicate why the teachings of Liu et al. and Stamps et al. do not support the Examiner's statement. Applicant points to Table 1 in Mathor et al. for support. In fact, Table 1 in Mathor et al. clearly demonstrates that results obtained with

one clone cannot be extrapolated to other clones. Specifically, Table 1 demonstrates that three different clones each comprising 3 copies of integrated vectors secrete different amounts of hIL-6 (180, 150, and 450 ng/10⁶ cells/day, respectively); two different clones comprising 4 copies of integrated vectors also secrete different amounts of hIL-6 (522 and 449 ng/10⁶ cells/day, respectively). Therefore, the Examiner's statement is supported by the art, including the data in Table 1 of Mathor et al. Based on these teachings, one of skill in the art would expect clones with the same number of copies to have different expression levels, and therefore, would know to select multiple clones and look for the high expressing ones. In fact, as indicated in the rejection above, the prior art does teach obtaining stable host cells comprising 20 integrated copies of retroviral vectors. The prior art also teaches that the number of integrating events can be controlled by varying the MOI and that gene expression directly correlates with the number of integrated copies (see the rejection above). Based on the teachings in the art, one of skill in the art would have known how to introduce 20 or more copies of retroviral vector into a host cell genome. Therefore, the Examiner did not ignore the actual data and the statement that it would have been obvious and within the capabilities of one of skill in the art to obtain host cells with 20 or more integrated copies is not sheer speculation or unsupported extrapolation of the data by the Examiner.

Applicant argues that, since Zielske teaches that use of internal promoters such as CMV results in a plateau of expression at 4 copies per cell, is unexpected in view of the art. This is not found persuasive. Zielske teaches that the limit in transgene expression is related to the particular vector/promoter/transgene/host cell system used in his experiments (p. 926, paragraph bridging columns 1 and 2, p. 929, column 1, third paragraph). In support of these teachings, Schott et al. disclose that protein expression from retroviral vectors comprising an internal CMV promoter does not reach a plateau when increasing the copy number above 4. Moreover, the claims are not limited to the CMV promoter; they recite any internal promoter. The prior art teaches successful expression from internal promoters in general, including CMV, and therefore the claimed invention is not unexpected.

4. The Examiner first states that "apart from an argument, Applicant and his Declaration did not provide any evidence indicating that obtaining host cells with genomes comprising greater than 20 integrated viral vectors was not feasible before the instant invention was made." This simply is not true. The Declarations provided citations to papers that demonstrate why it was completely unexpected that stable, high-producing cell lines could be developed that contained greater than 20 integrated retroviral vectors. In further support of the fact that the claimed invention was not expected and was not predictable, I am attaching 18 scientific papers to this Declaration that demonstrate why, at the time we made this invention, it was unexpected and not predictable that making cells lines with greater than 20 integrated retroviral vectors would be successful. These papers demonstrate that at the time of the invention, the usefulness of retroviral vectors for expressing proteins was in great question because expression from the

vectors was often inactivated by methylation. The following references demonstrate this:

AKGUN et al, "Determinants of Retrovirus Gene Expression in Embryonal Carcinoma Cells," Journal of Virology, Jan. 1991, Vol. 65(1) P. 382-388

BESTOR TH et al, "Creation of genomic methylation patterns," Nature Genetics, 1996, Vol. 12(4) P. 363-7

CHALLITA et al, "Lack of expression from a retroviral vector after transduction of murine hematopoietic stem cells is associated with methylation in vivo," Proc. Natl. Acad Sci USA, March 1994, Vol. 91, P. 2567-2571

CHERRY et al, "Retroviral Expression in Embryonic Stem Cells and Hematopoietic Stem Cells," Molecular and Cellular Biology, Oct. 2000, Vol. 20(20) P. 7419-7426

ELLIS et al, "The beta-globin locus control region versus gene therapy vectors: a struggle for Expression" Clinical Genetics, Jan. 2001, Vol. 59(1) P. 17-24

GORMAN et al, "Negative Regulation of Viral Enhancers in Undifferentiated Embryonic Stem Cells," Cell, Sept. 1985, Vol. 42, P. 519-526

HOEBEN et al, "Inactivation of the Moloney Murine Leukemia Virus Long Terminal Repeat in Murine Fibroblast Cell Lines Is Associated with Methylation and Dependent on Its Chromosomal Position," Journal of Virology, Feb. 1991, Vol. 65(2), P. 904-912

JOLLY et al, "Variable Stability of a Selectable Provirus after Retroviral Vector Gene Transfer into Human Cells," Molecular and Cellular Biology, Apr. 1986, Vol. 6(4), P. 1141-1147

PALMER et al, "Genetically modified skin fibroblasts persist long after transplantation but gradually inactivate introduced genes," Proc. Natl. Acad. Sci. USA, Feb. 1991, Vol. 88, P. 1330-1334

PANNELL et al, "Genetically modified skin fibroblasts persist long after transplantation but gradually inactivate introduced genes," The EMBO Journal, 2000, Vol. 19(21), P. 5884-5894

PANNELL et al, "Silencing of gene expression: implications for design of retrovirus vectors," Reviews in medical Virology, 2001, Vol. 11, P. 205-217

NIWA et al, "Independent Mechanisms Involved in Suppression of the Moloney Leukemia Virus Genome during Differentiation of Murine Teratocarcinoma Cells," Cell, Apr. 1983, Vol. 32, P. 1105-1113

SINGAL et al, "Methylation of the minimal promoter of an embryonic globin gene silences transcription in primary erythroid cells," Proc. Natl. Acad. Sci. USA, Dec. 1997, Vol. 94, P. 13724-13729

SVOBODA et al, "Retroviruses in foreign species and the problem of provirus silencing," *Gene*, 2000, Vol. 261, P. 181-188

TURKER et al, "Formation of methylation patterns in the mammalian genome," *Mutation Research*, 1997, Vol. 386(2), P. 119-130

WANG et al, "High-Resolution Analysis of Cytosine Methylation in the 5' Long Terminal Repeat of Retroviral Vectors," *Human Gene Therapy*, Nov. 1998, Vol. 9(16), P. 2321-2330

Furthermore, the references indicate that the problem with inactivation was associated with increased copy number as demonstrated by these references:

GARRICK et al, "Repeat-induced gene silencing in mammals," *Nature Genetics*, Jan. 1998, Vol. 18(1), P. 56-9

METHITALI et al, "The methylation-free status of a housekeeping transgene is lost at high copy number," *Gene*, 1990, Vol. 91(2), P. 179-84

These problems were observed across cell types, including stem cells, hematopoietic stem cells, fibroblasts and teratocarcinoma cells. Thus, at the time of the invention, it would have not have been expected or predictable that making cells that contain greater than 20 copies of a retroviral vector would be useful expressing proteins. At the time of the invention, based on the references cited above, it would have been expected that increasing the copy number of retroviral vectors past 20 would result in methylation, inactivation and instability.

5. Then the Examiner supports her conclusions that the claimed invention is expected by making arguments that mischaracterize the cited references and interpret and extrapolate the data in ways that are scientifically improper.

6. First, the Examiner states that:

It is noted that Table 1 (to which Applicant refers to) shows the result obtained with only one keratinocyte clone for each 8 and 15 integration events. Such results cannot be extrapolated to all clones. The Examiner provided evidence to support this statement and it is not clear why Applicant argues that such a statement is sheer speculation and an unsupported extrapolation of the data. It is well established in the art that retroviral insertion is random and that expression level is dependent on the insertion sites (see Mathor et al., of record, p. 10376, column 1; Liu et al., of record, *Anal Biochem*, 2000, 280: 20-28, Abstract, p. 21, column 1; Stamps et al., of record, *Int J Cancer*, 1994, 57: 865-874, Abstract, p. 868, column 1, p. 869, Fig. 2). It is noted that Applicant did not

indicate why the teachings of Liu et al. and Stamps et al. do not support the Examiner's statement. Applicant points to Table 1 in Mathor et al. for support. In fact, Table 1 in Mathor et al. clearly demonstrates that results obtained with one clone cannot be extrapolated to other clones. Specifically, Table 1 demonstrates that three different clones each comprising 3 copies of integrated vectors secrete different amounts of hll-6 (180, 150, and 450 ng/10⁶ cells/day, respectively); two different clones comprising 4 copies of integrated vectors also secrete different amounts of hll-6 (522 and 449 ng/10⁶ cells/day, respectively). Therefore, the Examiner's statement is supported by the art, including the data in Table 1 of Mathor et al. Based on these teachings, one of skill in the art would expect clones with the same number of copies to have different expression levels, and therefore, would know to select multiple clones and look for the high expressing ones.

The issue here is what Table 1 of Mathor shows to one of skill in the art and in particular whether the data in Mathor indicates to one of skill in the art that cells lines with greater than 20 integrated retroviral vectors would yield higher expression levels than cells lines with 8 or 15 integrated retroviral vectors. The data in Table 1 of Mathor, which is limited to a maximum of 15 integrations, cannot be extrapolated to a situation where there are 20 integrations. It is impossible to do a valid statistical analysis or curve fit based on the data in Mathor. To provide any other interpretation to the data is not scientifically correct. For example, as shown in Appendix 1, the data could indicate a plateau or upside-down U shaped curve as shown. This is why I have previously said it was not proper to extrapolate the data to cells that have 20 integrations. A person of skill in the art would not do this.

In response, the Examiner has argued that "It is well established in the art that retroviral insertion is random and that expression level is dependent on the insertion sites (see Mathor et al., of record, p. 10376, column 1; Liu et al., of record, Anal Biochem, 2000, 280: 20-28, Abstract, p. 21, column 1; Stamps et al., of record, Int. J Cancer, 1994, 57: 865-874, Abstract, p. 868, column 1, p. 869, Fig. 2). While this may be true, it is not relevant to why the data of Mathor can be extrapolated to support the Examiner's conclusion and what that data indicates to one of skill in the art. The person of skill in the art would interpret that data as shown in Appendix 1, especially in light of the knowledge of methylation and gene silencing, as indicating that expression was declining or plateauing with increasing copy number. In particular, neither Lui nor Stamps provide any information that can be used to predict whether a cell line containing 20 integrated retroviral vectors will produce more protein than a cell line with 8 or 15 integrated retroviral vectors. For this reason, citation of Liu and Stamps provides information which is not scientifically relevant and do not support a scientifically valid argument.

The Examiner further relies on data in Mathor that shows that "three different clones each

comprising 3 copies of integrated vectors secrete different amounts of hIL-6 (180, 150, and 450 ng/10⁶ cells/day, respectively); two different clones comprising 4 copies of integrated vectors also secrete different amounts of hIL-6 (522 and 449 ng/10⁶ cells/day, respectively). It is certainly expected that different cell lines with the same number of integrations would have different expression levels. However, this has no relevance or predictive value with respect to the issue of whether, according to the data in Table 1, a cell line with 20 integrated retroviral vectors would produce more protein than a cell line with 8 or 15 integrated vectors. Indeed, based on the data, the curve appears to be declining or at best plateauing. No other prediction can be made from the data.

7. The Examiner further states that:

Applicant argues that, since Zielske teaches that use of internal promoters such as CMV results in a plateau of expression at 4 copies per cell, is unexpected in view of the art. This is not found persuasive. Zielske teaches that the limit in transgene expression is related to the particular vector/promoter/transgene/host cell system used in his experiments (p. 926, paragraph bridging columns 1 and 2, p. 929, column 1, third paragraph).

The Examiner's argument that Zielske teaching are limited to a particular vector/promoter/transgene system is also not relevant to the issue of whether a person of skill in the art would have predicted or expected that that cells lines with greater than 20 integrated retroviral vectors would yield higher expression levels than cells lines with 8 or 15 integrated retroviral vectors. The Examiner initially cited Zielske as support for an argument that the person of skill in the art would have been motivated to make cell lines containing multiple copies of an integrated retroviral vector. However, Zielske does not support making cell lines with 20 integrated vectors regardless of the vector/promoter/transgene system. I note that Mathor also teaches a plateau or expression maximum at 8 integrated copies with a different vector/promoter/transgene system. Thus, Mathor and Zielske have similar teaching that would discourage making cell lines that contain greater than 20 integrated retroviral vectors.

8. The Examiner next argues that:

In support of these teachings, Schott et al. disclose that protein expression from retroviral vectors comprising an internal CMV promoter does not reach a plateau when increasing the copy number above 4. Moreover, the claims are not limited to the CMV promoter; they recite any internal promoter. The prior art teaches successful expression from internal promoters in general, including CMV, and therefore the claimed

invention is not unexpected.

Schott does not teach a plateau at 4 copies, but only includes data to 9 copies. Again Schott is not relevant to the issue of whether a person of skill in the art would have predicted or expected that that cells lines with greater than 20 integrated retroviral vectors would yield higher expression levels than cells lines with 8 or 15 integrated retroviral vectors. Based on a combination of Zielske, Mathor and the other references I provided above, one would expect expression to decline or plateau.

9. The Examiner further argues that:

In fact, as indicated in the rejection above, the prior art does teach obtaining stable host cells comprising 20 integrated copies of retroviral vectors. The prior art also teaches that the number of integrating events can be controlled by varying the MOI and that gene expression directly correlates with the number of integrated copies (see the rejection above). Based on the teachings in the art, one of skill in the art would have known how to introduce 20 or more copies of retroviral vector into a host cell genome. Therefore, the Examiner did not ignore the actual data and the statement that it would have been obvious and within the capabilities of one of skill in the art to obtain host cells with 20 or more integrated copies is not sheer speculation or unsupported extrapolation of the data by the Examiner.


The reference cited by the Examiner to show 20 integrated copies, Persons et al., is directed to packaging cell lines for the production of infectious retroviral vectors, and thus is not relevant to using retroviral vectors to transduce cells to make a protein of interest. Persons et al. does not not address protein production or the impact of including multiple copies of a retroviral vector in a cell line for protein production.

10. The Examiner also argues that the teachings of Bestor relate to silencing in vivo, not in vitro. This statement is untrue as many of the references cited by Bestor and known and available to those of skill in the art clearly show this effect in vitro. In this regard, the Examiner is referred to the references cited above in Paragraph 4. Furthermore, the Examiner's arguments based on Persons (i.e., 20 copies of a vector in the genome with no silencing) are not relevant to the claims because Persons is directed to making retroviral packaging cells that produce infectious retroviral particles, not cell lines that are used for protein production.

11. I further declare that all statement made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of title 18 of the United States

Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Dated: September 17, 2009



Dr. Gregory Bleck